# Active Immunization Against Poliomyelitis*

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FOR the past 5 years, we have carried out experiments with a view toward developing a safe and effective method of immunization against poliomyelitis. In this paper, we propose to give a report of our approach and to present the data collected up to the present time.

Using *Macacus rhesus* monkeys, three methods were tried. These included the use of sub-infective doses of active virus, virus-serum combinations, and germicidally inactivated virus.

The use of active virus was, as one would expect, the most effective, but it proved dangerous, for some of the animals developed the disease during the course of immunization. Since, neither in poliomyelitis nor in other virus diseases is there any evidence for the belief that the virus loses its infectivity for man through animal passage, it was not deemed advisable to try it in the human.

Virus-serum combinations produced immunity but were difficult to standardize.

Therefore, germicidally inactivated virus was resorted to, inasmuch as the experiences of Bedson [1] and others with the viruses of herpes, psittacosis, fowl-plague, foot and mouth disease, and rabies, indicated that after chemical treatment, immunization could be obtained with non-infective preparations, whereas heat killed material was ineffective.

In a recent article Flexner [2] maintained that there was no evidence to show that germicidally inactivated virus engendered immunity. However, he refers to experiments involving too few animals to be significant. Moreover, in that work no significance was attached to the importance of treating the virus so as to render it non-infective, but not overtreating it. In our work it was found, in both monkeys and children, that when formalin acts upon the virus for the minimum amount of time necessary to render it non-infective, the material is superior to virus that is overtreated. This is in keeping with similar findings by Bedson and others for foot and mouth virus.[3] In fact, as we will point out, storage for more than 2 to 3 weeks at icebox temperature, which allows overaction of the formalin, renders the vaccine non-antigenic. Moreover, with germicidally inactivated viruses, as has been shown by the work of MacKenzie [4] and others, it is necessary to use large doses. In both monkeys and humans we found this to be the case in immunization against poliomyelitis.

With freshly prepared material, inactivated for just the proper length

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of time, and given in large doses, we have been able to demonstrate serum antibody in monkeys. Likewise, both Olitzky[5] of the Rockefeller Institute, and Schultz[6] of Stanford University have reported antibody production in monkeys with formalized vaccine. They failed to demonstrate any resistance to intracerebral inoculation with their test, which was more severe than we used for the demonstration of tissue immunity. We have reported that even large amounts of active virus given to monkeys immunized them against only small or moderate intracerebral doses of virus. The method for demonstrating such a fine degree of immunity has been to carry out simultaneous titrations of the virus in a series of 8 monkeys, putting 2 animals on each of 4 doses. The immunized animals receive 1 to 3 times the smallest amount of virus that produces complete paralysis in both monkeys in the usual incubation period.

When serums are tested, the neutralizing power is estimated as the number of minimal completely paralyzing doses that the serum neutralizes—many times the smallest dose that brings down the controls. That such a method gives reasonable accuracy is indicated from the following results of titration:

During the time the monkey experimental work was being carried out, 94 animals were used in titrating the virus. Of 60 monkeys which received the minimal, or 2 to 4 times the minimal, completely paralyzing doses, 57 came down with complete paralysis in the usual incubation period. Only 2 of the other 34, which received less than the estimated infective dose of virus, succumbed to the disease.

During the past 16 months, because of the large number of monkeys used, it has not been possible to keep them for a long enough time prior to inoculation to get them on uniform rations and to weed out those with intercurrent infection. As a result, the virus titrations have not been so constant, and, to offset this, sera are tested prior to receiving vaccine on 5 to 10 infective doses respectively, and multiples thereof. After vaccination the sera are tested against 40 and 80 infective doses and multiples thereof. The test for antibody in humans, therefore, is sufficiently coarse to meet the criteria of antibody in the hands of others.

Like Flexner, who has pointed out that there is no evidence to believe that monkey passage virus is non-infective for man, we have felt the use of non-infective material the safest procedure and therefore worthy of extensive trial to determine whether or not whatever immunity it produced would be sufficient to protect children against the disease. The safety of the vaccine is indicated by the fact that intracerebral and intraperitoneal inoculations have failed to infect monkeys. In addition, the formalin in the concentration used renders non-infective chorio-meningitis, herpes, and other viruses, and so should render inert any chance virus the monkey may carry. We realize that should active immunization protect against the disease, it will not completely solve the problem of poliomyelitis because of the difficulty of vaccinating so many in the absence of a susceptibility test. However, we do not agree with Aycock and others who maintain that the procedure is unwarranted because of the relatively small number of susceptible individuals. To maintain that infection with the virus of poliomyelitis is a question of susceptibility entirely, is an assumption, as we will point out later.

Those most likely to contract poliomyelitis are children below 10 years of age. The greatest incidence of the disease is in the 1–5 year group and in the 6–10 year old group, which is in keeping with the larger proportion in these groups who show no antibody,

and the low average level of such a group. This is indicated in Table I where the amount of antibody is designated as none, where the 0.9 c.c. of serum neutralizes less than 10 infective doses of virus, slight amounts, where it neutralizes 10–50 infective doses, which is probably an almost negligible amount, moderate, neutralizing 50–200 infective doses; and considerable amounts in which more than 200 infective doses are neutralized. Thus it would seem that the lack of antibody is a factor predisposing to the disease inasmuch as over 85 per cent of those under 5, and over 70 per cent of the 6–10 year old group show no antibody or only a small amount of antibody.

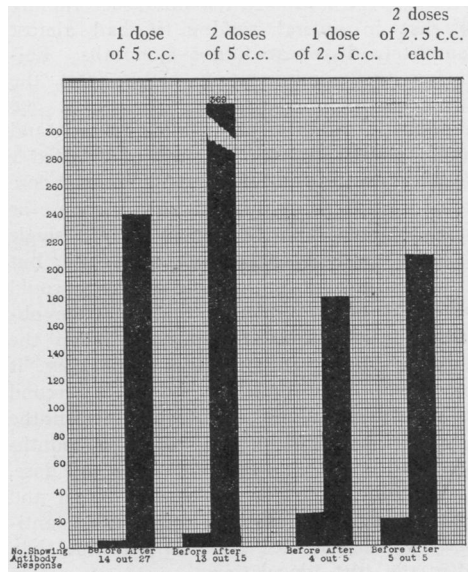
This does not explain why in an epidemic approximately only 1 of the 170 children under 5 showing no antibody, and about the same proportion of those under 10 develop the disease. This may be due to the individual non-specific variation in the susceptibility of the children, or the size of the infective dose, or number of exposures, or the accessibility of the virus to the olfactory fila.

Until these or other factors in the epidemiology of the disease are understood, then mass immunization, though wasteful, is a logical procedure.

### IMMUNIZATION OF CHILDREN

This study consists of 2 parts, (1) the determination of antibody in approximately 150 children, and (2) field studies.

GRAPH I—Antibody Response to one and two doses—5 c.c. or 2.5 c.c.




### ANTIBODY RESPONSES

These may be summarized as follows:

1. The immune response was proportional to the size of the dose tried for as Graph I indicates, with 5 c.c. doses the response was decidedly better than with 2.5 c.c. amounts.

2. As would be expected, where antibody was already present the response was decidedly better and in keeping with the amount of natural antibody present.

3. In the absence of any natural antibody, 60 to 65 per cent responded to a single, and nearly 90 per cent to 2 doses. Throughout, 2 doses gave a greater incidence, degree and duration of immunity than did 1 dose, as is indicated in Graphs I, II, III, and IV. In Graphs II and III are recorded the data collected from tests done on the serum of a small group of children at various intervals

TABLE I

NORMAL URBAN ADULTS

| | *No Antibody Neutralizes Less 10 M.C.P.* | *Slight Antibody Neutralizes 10–50 M.C.P.* | *Moderate Antibody Neutralizes 60–200 M.C.P.* | *Considerable Antibody Neutralizes 200+ M.C.P.* | *Total* |
|---|---|---|---|---|---|
| 6 mo.– 1 yr. | 5 | 2 | | | 7 |
| 2 yr.– 5 yr. | 54–62.8% | 19–22.1% | 11–12.8% | 2– 2.3% | 86 |
| 6 yr.–10 yr. | 15–38.5% | 13–33.3% | 9–23.1% | 2– 5.1% | 39 |
| 11 yr.–17 yr. | 0 | 1– 6.25% | 5–31.25% | 10–62.5% | 16 |
| Adults | 1– 2.6% | 2– 5.3% | 6–16% | 28–76% | 37 |

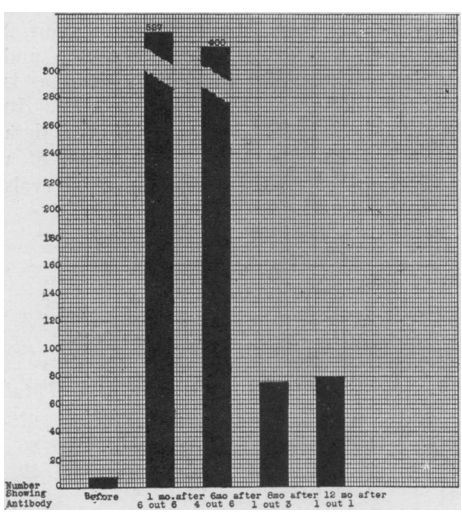
after immunization. The group which received a single dose responded exceedingly well, but the antibody fell off rather rapidly so that in several children it had almost completely disappeared in 5–8 months. Following the administration of 2 doses the antibody produced was still present at 5–8 months in all the individuals tested and could be demonstrated in 4 out of 6 tested 1 year after immunization. Where 1 dose failed, a second often produced a response as indicated in Graph IV, where after a single dose no antibody developed in a child, but after the second considerable antibody could be demonstrated. Similar findings were obtained in 6 out of 7 children. When the antibody disappeared after a single dose, it sometimes responded rapidly to a second dose. Three children from whose serum the antibody had disappeared within 6 months after the first dose were given a second dose, and a fourth child was given a second and third dose. All showed considerable antibody which persisted for a longer time than after the first inoculation.

Upon using brain stem as well as cord for preparation of the vaccine it was inactivated in a shorter period of time. Several series of children were given different batches of vaccine incubated with formalin for different periods. Although the groups were small, the results given in Graph V indicate that with the longer exposures to formalin less antibody develops.

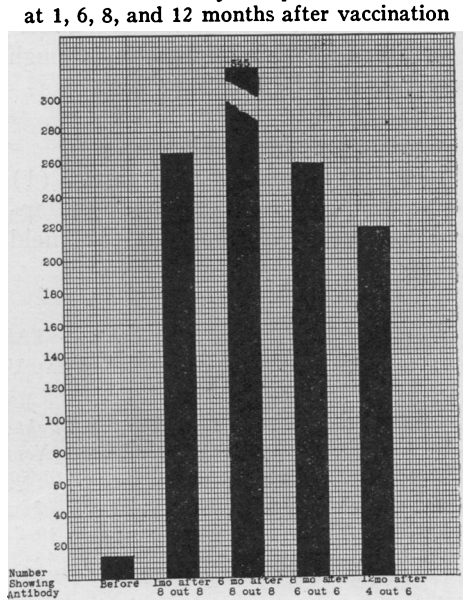
The vaccine does not keep well in storage. It was shown both in humans and monkeys that after storage for 1 month the vaccine produced but little antibody, and that after 2½ months at icebox temperature no antibody response could be demonstrated. It is interesting to note that at Seaview Hospital, New York, 1 of 4 children given vaccine which had been stored for 2½ months and who showed no antibody after immunization, contracted poliomyelitis. At this institution none of the 39 children given freshly prepared material developed the disease, whereas 1 of the non-vaccinated children came down.

Antibody can be demonstrated about 1 week after injection and reaches its height in 3 to 4 weeks. Therefore, no protection can be expected unless the person has received the vaccine at least 3 weeks prior to exposure. Thus, the

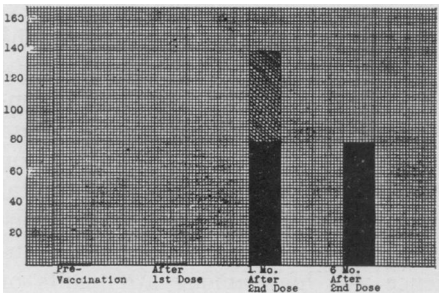
GRAPH II—Antibody Response—Single dose at 1, 6, 8, and 12 months after vaccination




GRAPH III—Antibody Response—Two doses at 1, 6, 8, and 12 months after vaccination



GRAPH IV—One Dose—No Response. A Second Dose—Antibody Response

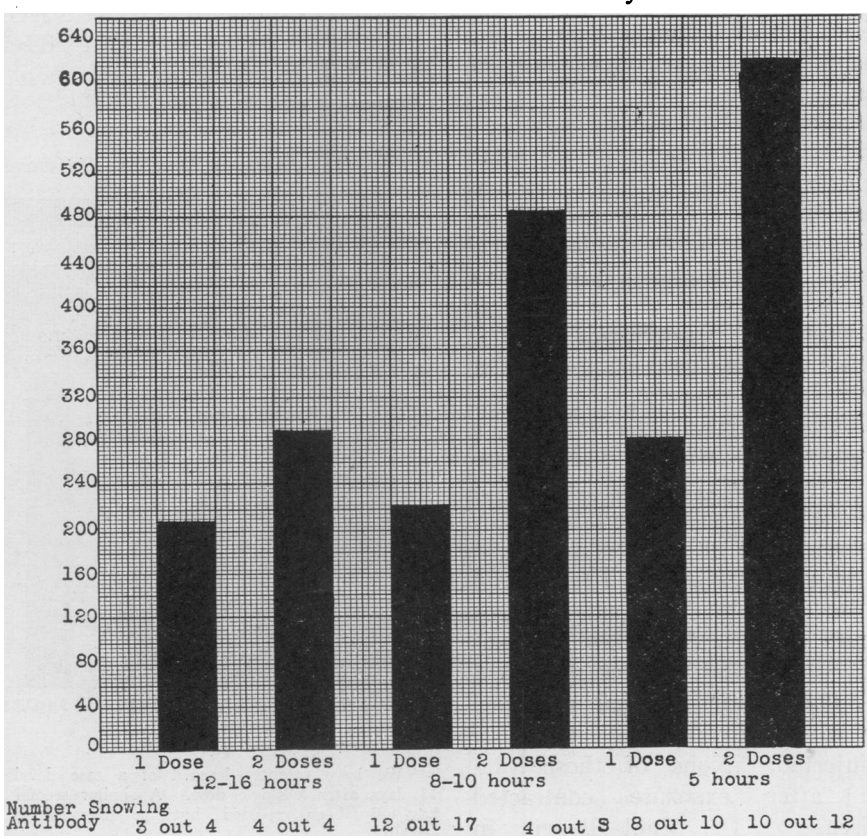



vaccine will be of no value where exposure has already taken place.

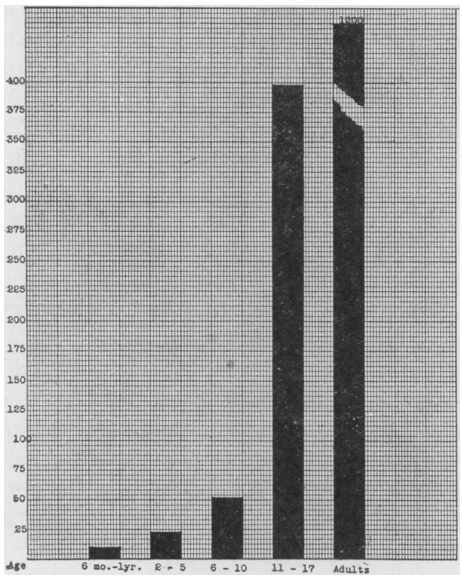
FIELD STUDIES

Up to the present more than 9,000 individuals have received vaccine. Inasmuch as more than 7,000 of these were in endemic or epidemic foci, it is quite likely that they were exposed to the virus. The control group, of non-vaccinated children consists of about 7,500. Of these approximately 4,500 were in exposed areas and can be compared with the similar group of 7,000 vaccinated. In so far as possible each vaccinated individual was matched with a control in the same district and of the same age. Wherever possible playmates were used. The details of the method of control have been discussed previously. Less than 1 per cent of those given the vaccine developed a general reaction. In cases where reaction did occur, it usually passed off in less than 24

GRAPH V—Antibody Response to Various Periods of Inactivation in Children with Little or No Pre-vaccinal Antibody



GRAPH VI—Antibody Levels in Normals



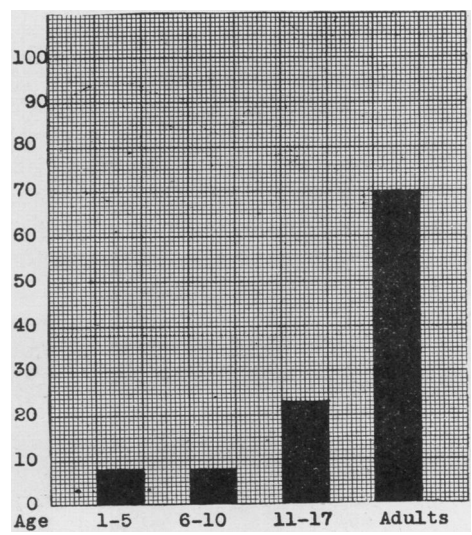

hours. About 2 per cent had local reactions such as induration and slight superficial necrosis, or occasionally a small abscess.

The vaccinated group included 564 individuals who were vaccinated after a definite history of exposure. Three of these developed the disease on the day of injection, and 1, 13 days later. The latter developed as a fulminating bulbar case. Inasmuch as it takes at least 3 weeks to obtain the full response from the vaccine, one cannot expect protection after exposure.

Of those vaccinated prior to exposure the largest groups were in North Carolina; Kern County, Calif.; and at the Los Angeles County hospital. The first group will be reported upon in detail by Dr. Gilliam. The second group has been reported on by Drs. Joe Smith and M. A. Gifford in their annual report, at which time somewhat over 3,000 had received vaccine. Nearly an additional 1,000 have since been injected. None of those vaccinated after exposure contracted the disease.* Dr. Emil Bogen, in reviewing the statistical data, states that, judging from the general incidence of the disease, one would have expected 3 to 5 cases to occur in the vaccinated group. In the town of Taft, where 652 were vaccinated and controls set up, 2 in the control group contracted the disease. Of the 200 on the staff of the Kern County hospital who received the vaccine, none contracted the disease, although the incidence was high among attendants of neighboring hospitals, such as those of Los Angeles County, Orange County, and Fresno County. Since all of the hospital staff were vaccinated, there were no controls for evaluating the vaccine.

At the Los Angeles County Hospital, where more than 300 nurses and physicians were vaccinated, Dr. Kessel reported that in one group of 42 vaccinated (15 received Kolmer vaccine) none came down with the disease, whereas 3 of the control group of 50 contracted poliomyelitis.

GRAPH VII—Antibody Levels in Poliomyelitis




* We have recently learned of a case developing 13 days after a single dose. A similar second case has only been reported, but the diagnosis is not certain.

## DISCUSSION

These studies have brought out the fact that a formalized vaccine produces some antibody in both monkeys and humans. That antibody is an indication of some real immunity is based upon the following evidence:

1. In keeping with the findings of others, we have shown that the incidence and degree of antibody (Table I, Graph VI) increases with age, which correlate with the lower incidence of the disease in the higher age groups, so it would appear that the presence of this antibody is a factor in protecting older children and adults from the disease.

2. In comparing the immunity developed after the injection of active virus with that following the administration of formalized virus, it was found that with living virus the degree of both tissue and humoral immunity was higher.

3. The antibody content of a group of paralytic poliomyelitis cases was tested during the first week of the disease and compared with that in normal individuals. The presence and amount of antibody were compared for different age groups. It was found in the small group tested that persons with acute poliomyelitis usually had little or no antibody. Inasmuch as the level of antibody seems decidedly lower in poliomyelitis than in so-called normal individuals, as demonstrated in Graphs VI and VII, we feel that the lack of specific antiviral substance is one of the determining factors that predisposes individuals to poliomyelitis.

When the virus enters the nose, the antibody present in the nasal secretions, if in sufficient quantity, may neutralize the virus. Should this, the first line of defense, fail, then the virus can get to the central nervous system, where it may or may not multiply, depending upon the resistance of the cerebrospinal axis.

The combined humoral and tissue immunity of children can be tested only in following up the outcome of natural exposure. Up to the present, none * of nearly 7,000 who were probably exposed, after vaccination with fresh vaccine was completed, have contracted the disease, whereas 5 of a smaller control group have come down.

The field trials must continue on a still larger scale to give the final answer. We feel that further work in this direction is merited because:

1. The vaccine is non-infective upon intracerebral inoculation into monkeys and from all experiences to date appears to be harmless.

2. Some immunity is developed and although evidenced only as antibody production, we have indicated its importance in the mechanics of protection.

3. Immunization against other virus diseases such as rabies, encephalomyelitis and louping-ill, has been obtained under field conditions with similar types of vaccine.

## CONCLUSIONS

A formalized poliomyelitis vaccine which fails to infect monkeys after intracerebral inoculation and so probably is safe, stimulates antibody.

The present inadequate data have been favorable, and do not show that the vaccine does not immunize.

Field studies on at least 50,000 more children should continue in order to reach a positive evaluation.

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